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RASSB/Antimicrobials Division (7510C)

# DATA EVALUATION RECORD

**STUDY TYPE:** 90-Day Oral Toxicity [gavage]-[rat]; OPPTS 870.3100 [§82-1]; OECD 408.

**DP BARCODE:** D301664 **DECISION No.** 217014
**PC CODE:** 004115 **REGISTRATION No.** 59825R

**TEST MATERIAL (PURITY)**: Tetraacetylethylenediamine (TAED, min 99%).

**SYNONYMS:** N,N'-1,2-ethanediylbis[N-acetylacetamide], N,N'-ethylenebis[N-acetylacetamide] TAED

**CITATION:** Wolfe G., S. Borst (2000). 90-day oral toxicity study of tetraacetylethylene-diamine (TAED) in Sprague-Dawley rats. TherImmune Research Corporation (Gaithersburg, MD). TherImmune Study Number 1151-102, October 19, 2000. MRID 45299703. Unpublished.

**SPONSOR:** Warwick International Limited, Mostyn, Holywall, Flintshire CH89HE, United Kingdom.

## EXECUTIVE SUMMARY:

In a 90-day oral toxicity study (MRID 45299703) tetraacetylethylenediamine (TAED, >99% a.i.. batch/lot # 13405002) administered to 10 Sprague-Dawley rats/sex/dose by gavage in 1% carboxymethylcellulose at nominal dose levels of 0, 25, 500, or 1000 mg/kg bw/day.

There were no treatment-related findings regarding mortality, clinical signs of toxicity, ophthalmology, and hematology. Significant dose-related decreases in body weight and body weight gain were observed in treated males at all dose levels and ≥500-mg/kg/day in treated females. Food consumption was decreased in treated rats but the decreases reached statistical significance only in the 1000-mg/kg/day males. Significant increases occurred in total protein (1000-mg/kg/day males and females), albumin (1000-mg/kg/day males), and globulin values (1000-mg/kg/day females). Liver weights (absolute, relative to body and brain weights) were

significantly increased in the 1000-mg/kg/day males and ≥500-mg/kg/day females that correlated with incidences of grossly enlarged liver in 1000-mg/kg/day males and incidences of centri-lobular cytomegaly of the liver (1000 mg/kg/day males) in all 500- and 1000-mg/kg/day treated rats. Bilateral, mild degeneration of the seminiferous tubules was noted in 2/10 high-dose males.

**Based on weight loss and decrease in the rate of weight gain, microscopic lesions observed in the liver (centrilobular cytomegaly), increased liver weights, and clinical chemistry changes, the LOAEL is 500 mg/kg/day in females and 25 mg/kg/day in males. The NOAEL is 25 mg/kg/day in females, but could not be determined in males**

This 90-day oral toxicity study in the Sprague-Dawley rats is **Acceptable/Guideline**. The study satisfies the guideline requirement for a 90-day subchronic oral toxicity study [OPPTS 870.3100 [§82-1]; OECD 408] in rats.

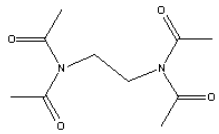
**COMPLIANCE:** Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

## I. MATERIALS AND METHODS

### A. MATERIALS:

**1. Test Material:** Tetraacetylethylenediamine (TAED)

| | |
|---|---|
| **Description:** | White powder |
| **Lot/Batch #:** | 13405002 |
| **Purity:** | > 99% |
| **Compound Stability:** | Stable |
| **CAS #:** | 10543-57-4 |
| **Structure** | |



(http://chemfinder.cambridgesoft.com/)

**2. Vehicle and/or positive control:** Carboxymethylcellulose, Lot # 108H0070 and 69H0153; purity was assumed to be 100% by the study laboratory.

**3. Test animals:**

| | | |
|---|---|---|
| **Species:** | Rat | |
| **Strain:** | CRL:CD(SD)IGS BR Sprague-Dawley | |
| **Age/weight at study initiation:** | ~7 weeks / males 191.7-232.7 g and females 150.8-181.6 g | |
| **Source:** | Charles River Laboratories, Inc. (Raleigh, NC) | |
| **Housing:** | Individually housed in suspended polycarbonate cages (9 x 8.5 x 8 in) with Sani Chip®, a heat sterilized hardwood bedding | |
| **Diet:** | TEKLAD™ Certified Rodent Diet 7012C *ad libitum*, except during fasting periods prior to blood collection and necropsy; analysis of nutrients and contaminants were performed and were reportedly on file at the study laboratory. | |
| **Water:** | Tap water by automatic watering system available *ad libitum*; contaminant and microbiological analysis were reportedly performed routinely and kept on file at the study laboratory. | |
| **Environmental conditions:** | **Temperature:** | 19-25° C |
| | **Humidity:** | 30-70% |
| | **Air changes:** | 10-15/hr |
| | **Photoperiod:** | 12 hrs dark/12 hrs light |
| **Acclimation period:** | 7 days | |

## B. STUDY DESIGN:

**1. In life dates** - Start: March 20, 2000; End: June 19 and 20, 2000

**2. Animal assignment**: Animals were assigned using a computerized random number generator to the test groups noted in Table 1.

**TABLE 1: Study design[a]**

| Test Group | Dose volume (mL/kg) | Dose to Animal (mg/kg/day) | # Male | # Female |
|---|---|---|---|---|
| **Control** | 5 | 0 | 10 | 10 |
| **Low** | 5 | 25 | 10 | 10 |
| **Mid** | 5 | 500 | 10 | 10 |
| **High** | 5 | 1000 | 10 | 10 |

[a] Data were obtained from p. 13 of the study report.

**3. Dose selection rationale:**

The basis for the dose levels utilized in the study was not stated.

**4. Test article preparation and analysis:**

Dose formulations were prepared weekly by mixing appropriate amounts of the test article with 1.0% carboxymethylcellulose (CMC). The mixture was stirred via magnetic stir bar for 30 minutes, then divided into daily aliquots and stored in amber vials at ~4°C. Homogeneity was evaluated in the first batch prepared of the low- (5 mg/mL) and high-dose formulations (200 mg/mL). Samples were taken from the top and bottom areas of 3 different strata. Additional samples from the low- and high-dose formulations were analyzed over a 10-day period (Days 0, 1, 4, 7, and 10) for stability at room temperature, refrigerated, and freezing temperature; actual temperatures were not provided. The concentrations of all dose formulations were analyzed on 3 occasions during the study at Weeks 1, 7, and 14.

**Results -**

**Homogeneity Analysis:** The results indicate that the samples were homogeneous. The low-dose formulation samples ranged from 89.4-101% of target concentration, and the high-dose formulation samples ranged from 95.6-98.4% of target concentration.

**Stability Analysis:** Table 2 provides a summary of the stability results. Except for 2 occurrences, stability was verified (within guideline standards of ±10%) in the low- and high-dose formulations under all storage conditions over the 10-day period. On Day 4, the low-dose formulation at room temperature was decreased 49% due to conglomeration of the test article which could not be resuspended. The analysis of the low-dose formulation at frozen temperature on Day 7 was 87% of Day 0; this decrease in concentration was not observed at the preceding measurement on Day 10 and, therefore, was not considered to be a significant deviation.

**TABLE 2. Stability analysis[a]**

| | Low Dose Formulation Mean±SD (% of Day 0) mg/mL | | | High Dose Formulation Mean±SD (% of Day 0) mg/mL | | |
|---|---|---|---|---|---|---|
| | **RT** | **Refr** | **Frozen** | **RT** | **Refr** | **Frozen** |
| Day 0 | 4.742±0.191 | | | 192.7±3.213 | | |
| Day 1 | 4.610±0.107 (97) | 4.440±0.078 (94) | 4.291±0.158 (91) | 180.45±5.445 (94) | 195.1±4.667 (101) | 195.9±3.677 (102) |
| Day 4 | 2.332±0.051[b] | 4.452±0.190 | 4.372±0.249 | 187.05±3.889 | 197.9±1.555 | 197.0±3.041 |

| | Low Dose Formulation Mean±SD (% of Day 0) mg/mL | | | High Dose Formulation Mean±SD (% of Day 0) mg/mL | | |
|---|---|---|---|---|---|---|
| | **RT** | **Refr** | **Frozen** | **RT** | **Refr** | **Frozen** |
| | (51) | (94) | (92) | (97) | (103) | (102) |
| Day 7 | 4.602±0.182 (97) | 4.441±0.050 (94) | 4.102±0.062 (87) | 190.1±2.546 (99) | 197.3±2.90 (102) | 195.1±0.071 (101) |
| Day 10 | 4.608±0.089 (97) | 4.419±0.029 (93) | 4.502±0.078 (95) | 190.0±3.394 (99) | 195.4±2.758 (101) | 206.3±8.91 (107) |

[a] Data derived from pp. 325-328 of the study report, the mean and standard deviations were calculated. RT: room temperature; Refr: refrigerated; Frozen. The low-dose target formulation was 5 mg/mL and the high-dose target was 200 mg/mL.
[b] The test article precipitated out of solution and created a conglomeration of material that could not be resuspended even after sonication.

**Concentration Analysis:** All but one sample was found to be outside the acceptable limit of ±10% of nominal concentration. The percent of the nominal concentration for mixtures on weeks 1, 7, and 14 were as follows: 63, 63, and 67%, respectively, for the low-dose formulations; 72, 84, and 70%, respectively, for the mid-dose formulations and 95, 85, and 72%, respectively, for the high-dose formulations. A retest was not performed nor was there any mention of this deviation in the study report.

The analytical data indicated that the mixing procedure was adequate and the dose formulations were stable up to 10 day at room temperature, refrigerated, or frozen. However, when concentration analyses were performed, the variance between nominal and actual dosage to the animals was not acceptable.

**5. Statistics** - Homogeneity of variance was cslculated in mean body weight, body weight gain, food consumption, hematology, clinical chemistry, and organ weight data. An ANOVA and Dunnett's T-test was subsequently used to compare the data at the 5% one tailed probability level.

## C. METHODS:

### 1. Observations:

1a. Cageside Observations

Animals were inspected twice daily, at least 6 hours apart, for signs of toxicity and mortality.

1b. Clinical Examinations

Clinical examinations were conducted at randomization and weekly thereafter.

1c. Neurological Evaluations

Cageside observations included the autonomic and central nervous systems, and somatomotor and behavior patterns. Functional observations were not performed.

**2. Body weight:**

Animals were weighed at randomization, prior to dosing, and weekly thereafter.

**3. Food consumption and compound intake:**

Food consumption for each animal was determined weekly and mean daily diet consumption was calculated as g food/kg body weight/day.

**4. Ophthalmoscopic examination:**

Eyes were examined in all animals prior to dosing, and in the control and high-dose animals prior to termination. Examinations included inspection of the anterior portion, optic media, and ocular fundus.

**5. Hematology & Clinical Chemistry:**

Blood was collected from fasted animals at study termination via the orbital sinus in carbon dioxide-anesthetized animals for hematology and clinical chemistry from all surviving animals. The CHECKED (X) parameters were examined.

**a. Hematology**

| | | | |
|---|---|---|---|
| X | Hematocrit (HCT)* | X | Leukocyte differential count* |
| X | Hemoglobin (HGB)* | X | Mean corpuscular HGB (MCH)* |
| X | Leukocyte count (WBC)* | X | Mean corpusc. HGB conc.(MCHC)* |
| X | Erythrocyte count (RBC)* | X | Mean corpusc. volume (MCV)* |
| X | Platelet count* | | Reticulocyte count |
| | Blood clotting measurements* | X | Mean platelet volume |
| X | (Thromboplastin time, APTT) | X | Cellular morphology |
| | (Clotting time) | | |

| | | |
|---|---|---|
| | (Prothrombin time) | |

* Recommended for 90-day oral rodent studies based on Guideline 870.3100.

## b. Clinical Chemistry

| | ELECTROLYTES | | OTHER |
|---|---|---|---|
| X | Calcium | X | Albumin* |
| X | Chloride | X | Creatinine* |
| | Magnesium | X | Urea nitrogen* |
| X | Phosphorus | X | Total Cholesterol* |
| X | Potassium* | X | Globulins |
| X | Sodium* | X | Glucose* |
| | **ENZYMES** | X | Total bilirubin |
| X | Alkaline phosphatase (ALP)* | X | Total protein (TP)* |
| | Cholinesterase (ChE) | X | Triglycerides |
| | Creatine phosphokinase | | Serum protein electrophoresis |
| | Lactic acid dehydrogenase (LDH) | X | A/G Ratio |
| X | Alanine aminotransferase (ALT/also SGPT)* | | |
| X | Aspartate aminotransferase (AST/also SGOT)* | | |
| | Sorbitol dehydrogenase* | | |
| | Gamma glutamyl transferase (GGT)* | | |
| | Glutamate dehydrogenase | | |

* Recommended for 90-day oral rodent studies based on Guideline 870.3100.

## 6. Urinalysis

Urinalysis was not conducted.

## 7. Sacrifice and Pathology

All animals that died and those sacrificed via carbon dioxide asphyxiation and exsanguination on schedule were subjected to gross pathological examination. The CHECKED (X) tissues were collected for histological examination from the controls and 1000-mg/kg/day treated animals except the liver was inspected in all treatment groups. The (XX) organs, in addition, were weighed.

| | DIGESTIVE SYSTEM | | CARDIOVASC./HEMAT. | | NEUROLOGIC |
|---|---|---|---|---|---|
| | Tongue | X | Aorta* | XX | Brain*+ |
| X | Salivary glands* | XX | Heart*+ | X | Peripheral nerve* |
| X | Esophagus* | X | Bone marrow* | X | Spinal cord (3 levels)* |
| X | Stomach* | X | Lymph nodes (mesenteric and mandibular)* | X | Pituitary* |

| | | | | | |
|---|---|---|---|---|---|
| X | Duodenum* | XX | Spleen*+ | X | Eyes (optic nerve )* |
| X | Jejunum* | XX | Thymus*+ | | **GLANDULAR** |
| X | Ileum* | | | XX | Adrenal gland*+ |
| X | Cecum* | | **UROGENITAL** | | Lacrimal gland |
| X | Colon* | XX | Kidneys*+ | X | Parathyroid* |
| X | Rectum* | X | Urinary bladder* | X | Thyroid* |
| XX | Liver*+ | XX | Testes*+ | | **OTHER** |
| | Gall bladder (not rat)* | XX | Epididymides*+ | X | Bone (sternum and/or femur) |
| | Bile duct (rat) | X | Prostate* | X | Skeletal muscle (gastrocnemius) |
| X | Pancreas* | X | Seminal vesicles* | X | Skin* |
| | **RESPIRATORY** | XX | Ovaries*+ | X | All gross lesions and masses* |
| X | Trachea* | XX | Uterus*+ | | |
| X | Lung* | X | Mammary gland* | | |
| X | Nose* | X | Fallopian tubes | | |
| X | Pharynx* | X | Vagina | | |
| X | Larynx* | | | | |

* Recommended for 90-day oral rodent studies based on Guideline 870.3100.
+ Organ weights required for rodent studies.

## II. RESULTS

### A. OBSERVATIONS:

**1. Clinical signs of toxicity** - No treatment-related findings were observed. Incidences of abrasions, alopecia, thinness, ulceration, and urine staining occurred sporadically.

**2. Mortality** - No animals died during the treatment period.

**3. Neurological Evaluations** - There was no evidence of neurological disturbances in the limited neurological observations made during the clinical examinations.

**B. BODY WEIGHT AND WEIGHT GAIN:** Body weight decreased in a dose-dependent manner throughout the study in treated males and females as depicted in Table 3. In males, significant decreases in body weight were observed at 25-mg/kg/day (Weeks 7-14), at 500-mg/kg/day (Weeks 4, 6-14), and at 1000-mg/kg/day (Weeks 2-14). There was a general decrease in body weight gain throughout the study in treated males, compared to controls; these decreases did not always reach statistical significance. The total body weight gain was statistically significantly decreased in all treatment groups, with % of control decreasing with increasing dose.

In females, significant decreases in body weight were observed at 500 mg/kg/day (Weeks 6-14) and at 1000 mg/kg/day (Weeks 5-14). Body weight gain was generally decreased throughout the study at ≥500-mg/kg/day, compared to controls; these decreases did not always reach statistical

significance. The total body weight gain was statistically significantly decreased in the 500- and 1000-mg/kg/day treatment groups, with % of control decreasing with increasing dose.

**TABLE 3. Average body weights and body weight gains during 90 days of treatment[a]**

| Dose rate mg/kg/day n=10 | Body Weights (g±SD) | | | | Total Weight Gain | |
|---|---|---|---|---|---|---|
| | Week 1 | Week 2 | Week 7 | Week 14 | g | % Difference [Control vs. treated] |
| **Male** | | | | | | |
| **0** | 215.20±11.60 | 277.89±14.41 | 478.52±30.68 | 593.03±48.18 | 377.83±43.34 | - |
| **25** | 214.39±12.23 | 266.25±19.87 | 433.02±40.91*+ | 530.94±59.20* | 316.55±57.18* | -16 |
| **500** | 216.28±9.46 | 266.14±12.17 | 416.99±24.07** | 482.35±52.86** | 266.07±54.72* | -30 |
| **1000** | 215.35±8.35 | 254.42±21.59* | 372.77±45.26** | 430.98±59.78** | 215.63±56.29* | -43 |
| **Female** | | | | | | |
| **0** | 164.82±6.96 | 189.21±7.61 | 269.07±9.15 | 313.47±14.76 | 148.65±12.11 | - |
| **25** | 162.99±9.08 | 186.18±12.01 | 273.19±21.97 | 305.15±27.14 | 142.16±25.25 | -4 |
| **500** | 162.23±6.40 | 183.76±9.30 | 249.97±12.83* | 276.80±15.09** | 114.57±14.52* | -23 |
| **1000** | 163.07±6.07 | 179.95±9.52 | 238.59±16.09** | 252.19±17.77** | 89.12±18.06* | -40 |

[a] Data obtained from pp. 25-26, and 28 in the study report.
[b] % of control.
* Statistically different ($p < 0.05$) from the control (Dunnett test).
** Statistically different ($p < 0.01$) from the control (Dunnett test).
*+ <10% vs. the control, Statistically different ($p < 0.05$) from the control (Dunnett test).

## C. FOOD CONSUMPTION AND COMPOUND INTAKE:

**1. Food consumption** - Food consumption was statistically significantly reduced in 1000-mg/kg/day males throughout the study. Decreased food consumption was also observed in 500-mg/kg/day males (Weeks 4-11), and in 25-mg/kg/day males (Weeks 6 and 12). As a result, food consumption at study termination was decreased 21% and 12% in the 1000- and 500-mg/kg/day

treated males, respectively, when compared to controls. Food consumption was statistically significantly decreased in 1000-mg/kg/day treated females at Week 5-6 only, with an 11% decrease at study termination, compared to controls.

**2. Compound consumption** - The test article was delivered by gavage and it was assumed that the dose was completely consumed. No incidences of regurgitation were noted.

## D. BLOOD ANALYSES:

**1. Hematology** -_Statistically significant decreases were observed in several hematology parameters (Table 4) including hemoglobin (500- and 1000-mg/kg/day females), hematocrit (500- and 1000-mg/kg/day females), mean cell volume (500-mg/kg/day males), and MCH (500- and 1000-mg/kg/day males). A statistically significant increase was observed in red blood cell count (1000-mg/kg/day males). Laboratory historical control data were not provided; however, historical control hematological ranges provided by Charles River (1993) for ~20 week old Sprague-Dawley rats indicated that these hematological changes were within the following historical control ranges: red blood cell count values of 7.13-9.75 $x10^6$/µL (males) and 6.69-8.99 $x10^6$/µL (females); hemoglobin values of 13.5-16.8 g/dL (males) and 13.3-16.4 g/dL (females); hematocrit values of 39-52.1% (males) and 39.9-51.6 % (females); mean cell volumes of 52-61 fL (males) and 53-62 fL (females); and MCH values of 16.7-19.7 pg (males) and 17.9-20.1 pg (females). The observed changes in hematology were not considered to be toxicologically significant because the differences were not related to dose, were of slight magnitude, were not consistent in both sexes. and/or were within historical control ranges.

**TABLE 4. Selected Hematology Parameters[a]**

| Observation | | Male | | | | Female | | | |
|---|---|---|---|---|---|---|---|---|---|
| mg/kg/day | | 0 | 25 | 500 | 1000 | 0 | 25 | 500 | 1000 |
| RBC mil/UL | MEAN<br>S.D.<br>% | 7.746<br>0.441<br>- | 7.923<br>0.424<br>+2.3 | 8.160<br>0.423<br>+5.3 | 8.217*<br>0.179<br>+6.1 | 7.421<br>0.222<br>- | 7.197<br>0.570<br>-3.0 | 7.043<br>0.45<br>-5.1 | 7.209<br>0.448<br>-2.9 |
| Hb g/dL | MEAN<br>S.D.<br>% | 15.35<br>0.66<br>- | 15.44<br>0.63<br>+0.59 | 15.46<br>0.48<br>+0.72 | 15.44<br>0.50<br>+0.59 | 15.65<br>0.41<br>- | 15.46<br>0.48<br>+0.72 | 14.93*<br>0.70<br>-4.6 | 14.73*<br>0.62<br>-5.9 |
| HCT % | MEAN<br>S.D.<br>% | 45.43<br>1.88<br>- | 46.23<br>2.08<br>+1.8 | 45.70<br>1.69<br>+0.59 | 46.60<br>2.42<br>+2.6 | 46.58<br>1.58<br>- | 45.12<br>2.49<br>-3.1 | 44.16*<br>2.21<br>-5.2 | 44.18*<br>1.83<br>-5.2 |
| MCV fL | MEAN<br>S.D.<br>% | 58.9<br>2.3<br>- | 58.4<br>1.8<br>-0.85 | 56.0*<br>2.1<br>-4.9 | 56.9<br>2.4<br>-3.4 | 62.8<br>2.1<br>- | 63.0<br>2.1<br>+0.32 | 62.9<br>2.0<br>+0.16 | 61.6<br>3.3<br>-1.9 |
| MCH pg | MEAN<br>S.D.<br>% | 19.83<br>0.72<br>- | 19.50<br>0.57<br>-1.7 | 18.97*<br>0.86<br>-4.3 | 18.78*<br>0.42<br>-5.3 | 21.11<br>0.78<br>- | 21.56<br>1.24<br>+2.1 | 21.23<br>0.76<br>+0.57 | 20.47<br>1.20<br>-3.0 |

[a] Data obtained from p. 31 in the study report (n=10, except 25-mg/kg/day females where n=9).

[b] % - Difference from control.
* Statistically different (p <0.05) from the control (Dunnett test).

**2. Clinical Chemistry** - Statistically significant decreases were observed in several clinical chemistry parameters (Table 5) including glucose (500- and 1000-mg/kg/day males and 1000-mg/kg/day females), creatinine (1000-mg/kg/day females), triglyceride (500- and 1000-mg/kg/day males), chloride (1000-mg/kg/day females), and aspartate aminotransferase (1000-mg/kg/day males and 500- and 1000-mg/kg/day females). Decreases in these parameters were not usually considered to be toxicological maniiftations.

Statistically significant increases were observed in total protein (1000-mg/kg/day males and females), cholesterol (500- and 1000-mg/kg/day females), albumin (1000-mg/kg/day males), and globulin (1000-mg/kg/day females).

**TABLE 5. Selected Clinical Chemistry Parameters[a]**

| Observation | | Male [mg/kg/day] | | | | Female [mg/kg/day] | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 25 | 500 | 1000 | 0 | 25 | 500 | 1000 |
| Glucose mg/dL | MEAN | 153.3 | 127.2 | 116.1* | 108.1* | 119.9 | 128.6 | 123.4 | 101.7* |
| | S.D. | 57.4 | 46.9 | 43.5 | 31.9 | 13.4 | 33.2 | 31.3 | 9.6 |
| | %[b] | - | -17 | -24 | -29 | - | +7.3 | +2.9 | -15 |
| Total Protein g/dL | MEAN | 7.43 | 7.47 | 7.86 | 8.06* | 8.01 | 8.20 | 8.62 | 9.04* |
| | S.D. | 0.34 | 0.45 | 0.33 | 0.51 | 0.61 | 0.33 | 0.62 | 0.61 |
| | % | - | +0.5 | +5.8 | +8.4 | - | +2.4 | +7.6 | +13 |
| AST IU/L | MEAN | 82.9 | 76.7 | 69.8 | 57.2* | 79.4 | 72.4 | 57.9* | 51.0* |
| | S.D. | 33.2 | 15.4 | 18.9 | 10.4 | 12.8 | 11.1 | 7.9 | 4.9 |
| | % | - | -7.5 | -16 | -31 | - | -8.8 | -27 | -36 |
| Chloride mmol/L | MEAN | 101.5 | 101.4 | 100.3 | 101.0 | 102.8 | 101.7 | 101.1 | 100.2* |
| | S.D. | 2.7 | 1.6 | 1.5 | 1.1 | 1.6 | 1.2 | 2.3 | 1.8 |
| | % | - | -0.10 | -1.2 | -0.49 | - | -1.1 | -1.7 | -2.5 |
| Cholesterol mg/dL | MEAN | 97.9 | 86.1 | 91.0 | 114.0 | 96.3 | 107.3 | 132.3* | 193.4* |
| | S.D. | 11.9 | 11.6 | 16.6 | 28.2 | 14.4 | 16.3 | 0.07 | 40.8 |
| | %[b] | - | -12 | -7.0 | +16 | - | +11 | +37 | +101 |
| Triglyceride mg/dL | MEAN | 116.0 | 88.1 | 54.7* | 48.7* | 69.6 | 66.9 | 67.6 | 67.0 |
| | S.D. | 58.1 | 35.2 | 9.8 | 13.6 | 22.7 | 19.3 | 35.3 | 26.5 |
| | % | - | -24 | -53 | -58 | - | -3.9 | -2.9 | -3.7 |
| Creatinine mg/dL | MEAN | 0.43 | 0.39 | 0.43 | 0.43 | 0.53 | 0.51 | 0.46 | 0.45* |
| | S.D. | 0.08 | 0.06 | 0.07 | 0.05 | 0.07 | 0.06 | 0.07 | 0.05 |
| | % | - | -9.3 | 0 | 0 | - | -3.8 | -13 | -15 |
| Albumin g/dL | MEAN | 4.73 | 4.70 | 4.86 | 5.14* | 5.59 | 5.76 | 6.08 | 6.07 |
| | S.D. | 0.25 | 0.22 | 0.25 | 0.32 | 0.65 | 0.38 | 0.46 | 0.55 |
| | % | - | -0.6 | +2.7 | +8.7 | - | +3.0 | +8.8 | +8.6 |
| Globulin g/L | MEAN | 2.7 | 2.77 | 3.00 | 2.92 | 2.42 | 2.44 | 2.54 | 2.97* |
| | S.D. | 0.3 | 0.33 | 0.18 | 0.30 | 0.13 | 0.19 | 0.27 | 0.34 |
| | % | - | +2.6 | +11 | +8.1 | - | +0.8 | +5.0 | +23 |

[a] Data obtained from p. 30 in the study report (n=10, except 25-mg/kg/day females where n=9).
[b] % - Difference from control. * Statistically different ($p < 0.05$) from the control (Dunnett test).

## E. SACRIFICE AND PATHOLOGY:

**1. Organ weight** - Absolute and relative weights for selected organs are depicted in Tables 6a (males) and 6b (females). Statistically significant increases were observed in absolute and relative liver weights. Absolute liver weight was increased in 1000-mg/kg/day males and ≥500-mg/kg/day females. Liver-to-body weight ratios were increased in both ≥500-mg/kg/day males and females, and liver-to-brain weight ratios were increased in 1000-mg/kg/day males and ≥500-mg/kg/day females. The increases in absolute and relative liver weights correlate with the increased incidence of centrilobular cytomegaly observed in the livers of these treated rats.

Other organ weight changes occurred without corresponding macroscopic and/or microscopic lesions and were attributed to significant weight loss in the mid- and high-dose treated rats. Significant decreases in absolute organ weight were observed in the spleen (≥500- mg/kg/day males), thymus (500-mg/kg/day males), epididymis (1000-mg/kg/day males), and brain (1000-mg/kg/day males). Significant increases in relative organ-to-body weights were observed in the heart (1000-mg/kg/day males and all treated females), ovaries (1000-mg/kg/day females), kidneys (all treated males and ≥500-mg/kg/day females), brain (all treated males and ≥500-mg/kg/day females), testes (≥25-mg/kg/day males), and adrenals (1000-mg/kg/day males and females). Significant decreases in relative organ-to- brain weights were observed in the spleen (1000-mg/kg/day males) and thymus (500-mg/kg/day males).

**TABLE 6a. Selected Absolute and Relative Organ Weights for Male Rats[a]**

| Dose rate mg/kg/day n=10 | Absolute (g) | | | Relative to Body Weight | | Relative to Brain Weight |
|---|---|---|---|---|---|---|
| | Body | Liver | Brain | Liver (%) | Brain (%) | Liver |

| | | Weight | | | | | |
|---|---|---|---|---|---|---|---|
| **0** | **MEAN** | 593.03 | 17.1531 | 2.2418 | 0.0289 | 0.0038 | 7.6678 |
| | **S.D.** | 48.18 | 1.7703 | 0.1076 | 0.0013 | 0.0003 | 0.8847 |
| | **%[b]** | - | - | - | - | - | - |
| **25** | **MEAN** | 530.94 | 15.4804 | 2.1947 | 0.0292 | 0.0042* | 7.0502 |
| | **S.D.** | 59.20* | 2.0471 | 0.0876 | 0.0027 | 0.0005 | 0.8540 |
| | **%** | -10 | -9.8 | -2.1 | +1.0 | +11 | -8.1 |
| **500** | **MEAN** | 482.35 | 18.3544 | 2.1759 | 0.0385* | 0.0046* | 8.4294 |
| | **S.D.** | 52.86** | 1.7704 | 0.1094 | 0.0062 | 0.0006 | 0.6068 |
| | **%** | -19 | +7.0 | -2.9 | +33 | +21 | +9.9 |
| **1000** | **MEAN** | 430.98 | 19.8426* | 2.1182* | 0.0459* | 0.0050* | 9.3554* |
| | **S.D.** | 59.78** | 3.3138 | 0.1280 | 0.0022 | 0.0007 | 1.4188 |
| | **%** | -27 | +16 | -5.5 | +59 | +32 | +22 |

[a] Data obtained from pp. 38-40 in the study report.

[b] % - Difference from control.

* Statistically different ($p < 0.05$) from the control (Dunnett test).

**Statistically different ($p < 0.01$) from the control (Dunnett test).

## TABLE 6b. Selected Absolute and Relative Organ Weights for Female Rats[a]

| Dose rate mg/kg/day n=10 | | Absolute (g) | | | Relative to Body Weight | | Relative to Brain Weight |
|---|---|---|---|---|---|---|---|
| | | **Body Weight** | **Liver** | **Brain** | **Liver (%)** | **Brain (%)** | **Liver** |
| **0** | **MEAN** | 313.47 | 8.2960 | 2.0078 | 0.0265 | 0.0064 | 4.1521 |
| | **S.D.** | 14.76 | 0.6643 | 0.1222 | 0.0020 | 0.0005 | 0.4853 |
| | **%[b]** | - | - | - | - | - | - |
| **25** | **MEAN** | 305.15 | 8.5534 | 1.9921 | 0.0280 | 0.0066 | 4.2959 |
| | **S.D.** | 27.14 | 1.0281 | 0.1274 | 0.0019 | 0.0006 | 0.4463 |
| | **%** | -2.7 | +3.1 | -0.78 | +5.4 | +3.1 | +3.5 |
| **500** | **MEAN** | 276.80** | 10.7615** | 1.9554 | 0.0389* | 0.0071* | 5.5116* |
| | **S.D.** | 15.09 | 0.9197 | 0.0898 | 0.0029 | 0.0005 | 0.5193 |
| | **%** | -12 | +30 | -2.6 | +47 | +11 | +33 |
| **1000** | **MEAN** | 252.19** | 12.5138** | 1.9607 | 0.0498* | 0.0078* | 6.4712* |
| | **S.D.** | 17.77 | 1.4004 | 0.1938 | 0.0060 | 0.0009 | 1.1929 |
| | **%** | -20 | +51 | -2.3 | +88 | +22 | +56 |

[a] Data obtained from pp. 38-40 in the study report.

[b] % - Difference from control.

* Statistically different ($p < 0.05$) from the control (Dunnett test).

**Statistically different ($p < 0.01$) from the control (Dunnett test).

**2. <u>Gross pathology</u>** - Enlargement of the liver was observed in 500- and 1000-mg/kg/day treated males. Table 7 provides the incidence data for macroscopic and microscopic hepatic observations. Other gross observations were not considered treatment-related based on low sporadic incidences or similar incidences in control and treated rats, and no supporting microscopic pathology.

## TABLE 7. Selected Incidences of Gross and Microscopic Pathology Data[a]

| Observation | Male [mg/kg/day] | | | | Female [mg/kg/day] | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 25 | 500 | 1000 | 0 | 25 | 500 | 1000 |
| **Macroscopic Findings of the Liver** | | | | | | | | |
| Enlarged | 0/10 | 1/10 | 1/10 | 4/8 | 0/10 | 0/10 | 0/10 | 0/10 |
| **Microscopic Findings of the Liver** | | | | | | | | |
| Centrilobular cytomegaly | 0/10 | 0/10 | 10/10 | 10/10 | 0/10 | 0/10 | 10/10 | 10/10 |

[a] Data obtained from pp. 286 and 290 in the study report.

**3. <u>Microscopic pathology</u>** - Treatment-related centrilobular cytomegaly of the liver was observed in all 500- and 1000-mg/kg/day males and females as indicated in Table 7. This finding correlates with the increased liver weight observed in these treatment groups.

Bilateral seminiferous tubule degeneration was also noted in 2/10 high-dose males. These males displayed a lower absolute and testes-to-body weight ratio as compared to the rest of the dose group, which displayed significantly increased relative weights.

## III. DISCUSSION and CONCLUSIONS

The concentrations of the active material were recalculated based on the concentration at the week 1 time points at all 3 dose levels.

Oral administration of TAED by gavage for 13 weeks did not result in significant or dose-related effects on clinical signs of toxicity, mortality, or hematology. Clinical chemistry findings of decreased glucose (≥500-mg/kg/day males and 1000-mg/kg/day females) and aspartate aminotransferase (1000-mg/kg/day males and ≥500-mg/kg/day females) were likely attributable to the weight loss observed in these treatment groups. Increases were also observed in total protein (1000 mg/kg/day males and females) , albumin (1000 mg/kg/day males), and globulin (1000 mg/kg/day females).

The microscopic liver changes noted in this study, centrilobular cytomegaly, were comparable to the findings in the dermal subchronic study (MRID 452743-05) with this compound. In this dermal study, both males and females dosed at 1000 mg/kg/day exhibited this histopathology. Based on the decreased body weight, the NOAEL could not be determined in male rats, and was 25 mg/kg/day in female rats. The LOAEL was 25 mg/kg/day in males and 500 mg/kg/day in females.

**IV. DEFICIENCIES:** A large variation existed in the concentration of the dosing solutions at the various time periods. In and of itself this does not affect the validity of the study.

**V. STUDY CLASSIFICATION:** This 90-day oral toxicity study in the Sprague-Dawley rats is **Acceptable/Guideline**. The study satisfies the guideline requirement for a 90-day subchronic oral toxicity study [OPPTS 870.3100 [§82-1]; OECD 408] in rats.

**VI. REFERENCE**

Charles River. (1993). Hematology Parameters for the Crl:CD®BR Rat. http://www.criver.com/techdocs/hematol/index.html.

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